ANTI-NP IMMUNOGLOBULIN GENE : SOMATIC HYPERMUTATION DOES NOT DIRECTLY INFLUENCE ITS SPECIFICITY CHANGE FROM HETEROCLITIC TO HOMOCLITIC

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ABSTRACT

It is generally accepted that in the maturation of an immune response, the quality of the antibody, as well as the quantity, will improve with time after immunization. In the mouse system, immunoglobulin obtained in the first days after immunization with NP-CGG showed greater specificity to NIP-CGG (heteroclitic specificity). While the specificity to the immunizing antigen (homoclitic specificity) was recovered few weeks later. Intensive study on its immunoglobulin structure and the sequences of its mRNA revealed that this change of specificity was due to the switch from kappa to lambda light chain rather than to the occurence of somatic hypermutation in the immunoglobulin gene.

In this paper, we describe the contribution of either somatic hypermutation or immunoglobulin light chain class switch to the change of specificity from heteroclitic to homoclitic.

INTRODUCTION

The analysis of antibodies produced by B cells from mice immunized with T cell-dependent antigen showed that at certain steps of B cell differentiation, somatic mutations are introduced into rearranged V region genes[1]. Many studies suggested that amino acid substitution in the variable regions of heavy (V_H) and light (V_L) chains arising from somatic mutation contribute to raising the affinity to antigen. On the other hand, mechanism of the specificity change with time after immunization is still not clearly elucidated. Soebandrio, et al. in 1987 showed the contribution of somatic hypermutation to the change of antibody recognition (specificity) during immunization with (4-hydroxy-3-nitrophenyl) acetyl (NP)[2]. Reth, et al. in 1977 observed that primary anti-NP sera of mice show heteroclitic characteristic, i.e. have higher affinity for the cross-reacting haptens (4-hydroxy-3,5-dinitro-phenyl) acetyl (NNP) and (4-hydroxy-5-iodo-3-nitro-phenyl) acetyl (NIP) than for the homologous hapten NP, as well as the presence of lambda, mu and gamma-1 chains[3]. In this paper we discuss our observation on the factors influence the specificity change from heteroclitic to homoclitic of anti-NP antibody.

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MATURATION OF IMMUNE RESPONSE

Soon after immunization by a TD antigen, B cells will proliferate and differentiate into antibody secreting cells, i.e. plasma cells, and memory cells. Upon continuous stimulation by antigen, the B cells will differentiate into plasma cells, which secret antibody with increasing affinity and specificity, as well as quantity. This phenomenon is referred to as the maturation of immune response[4]. As described earlier, after a single injection of NP-CGG, late anti-NP thus developed gained a much better affinity and specificity, in term of recognition of both forms of NP hapten[2,5]. From the other point of view, specificity can be defined as the ability of a given antibody to differentiate the immunizing antigen from cross reacting antigens, i.e. antigens with very similar structure. As shown in figure 1, we observed that early anti-NP antibody has higher affinity to NIP, while the late one recognizes NP, the immunizing antigen, much better. This means that during the immunization process, the specificity changed from heteroclitic to homoclitic. This result is in agreement with the finding of other investigators[4,6].

COMPOSITION OF EARLY AND LATE ANTI-NP Abs.

To confirm its properties, we studied the anti-NP at the molecular level by constructing a battery of early and late anti-NP monoclonal antibody (mAb). Figure 2 shows binding property of several anti-NP mAbs. Most of lambda bearing anti-NP Abs have heteroclitic characteristic, while kappa bearing anti-NP Abs are homoclitic. A similar result was also reported by other investigators[4,7]. From these observations, we suggested that the change of the anti-NP Ab from heteroclitic to homoclitic is strongly influenced by the ratio between the homoclitic and the heteroclitic antibody in the serum. Experimentally, we made mixtures of various ratios of kappa and lambda bearing anti-NP mAbs and then subjected to a binding assay, as we have done to the serum. As shown in figure 3, the specificity of the mixture changes from heteroclitic to homoclitic with the increase of ratio between kappa and lambda bearing antibodies.

ROLE OF SOMATIC MUTATION IN THE SPECIFICITY MATURATION

It has been shown that somatic mutation can contribute to the increase of ability of an anti-Np mAbs to recognize both configurations of NP hapten[2] and can change the specificity of a given antibody drastically[8]. How far can this somatic mutation contribute to the change from heteroclitic to homoclitic?

From several mAbs we constructed and studies on its affinity to NP and NIP hapten, heavy and light chain classes, and cDNA sequences, we observed that there is no direct relationship between somatic mutation of the immunoglobulin gene and the heteroclitic-homoclitic properties, as shown in the Table 1.

CONCLUSION

From the above observation, we conclude that the change of specificity from heteroclitic to homoclitic is not directly influenced by somatic mutation but directly related to the ratio between kappa bearing (which is homoclitic) and lambda bearing (which is heteroclitic) anti-NP antibodies. Further investigation still has to be carried out

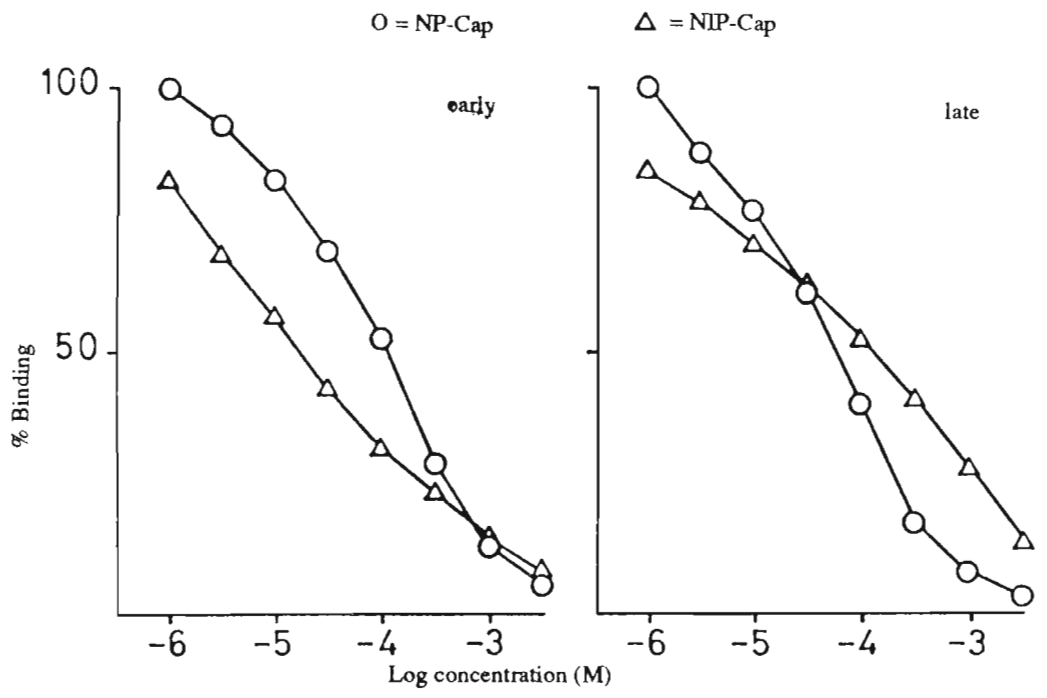

Figure 1. Inhibition assay of early and late anti-NP antibodies. 20 C57BL/6 Mice were immunized once with NP-CGG and serum collected at day 7,14,35,56, and 280 were subjected to an ELISA/RIA inhibition assay using either NP (represented by circles) or NIP (represented by triangles) as the inhibitting antigen. The early antibody shows heterocliticity while the late one is homoclitic. The crossing points between curves represent the percentage of homoclitic antibody in the serum, which is increasing with time after immunization.

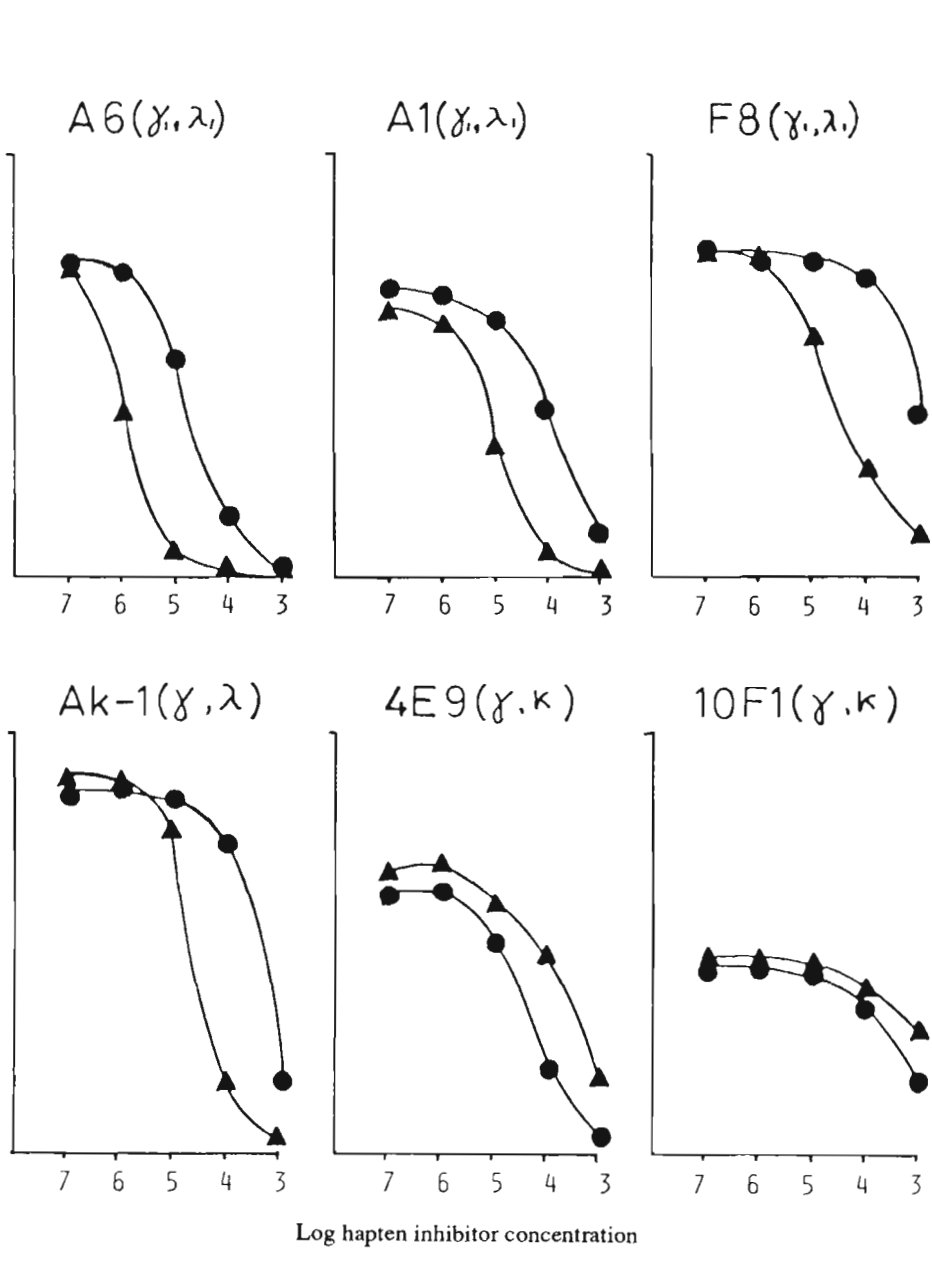

Figure 2. Inhibition assay of various anti-NP mAbs. Spleen cells of C57BL/6 mice immunized with NP-CGG were fused with SP2O.Ag.14 or X63.Ag8.653 parent cells at various stages of immunization. mAbs thus obtained were subjected to ELISA/RIA inhibition assay as described in Fig.1. Lambda bearing mAbs show heteroclitic property, while the kappa bearing ones are homoclitic.

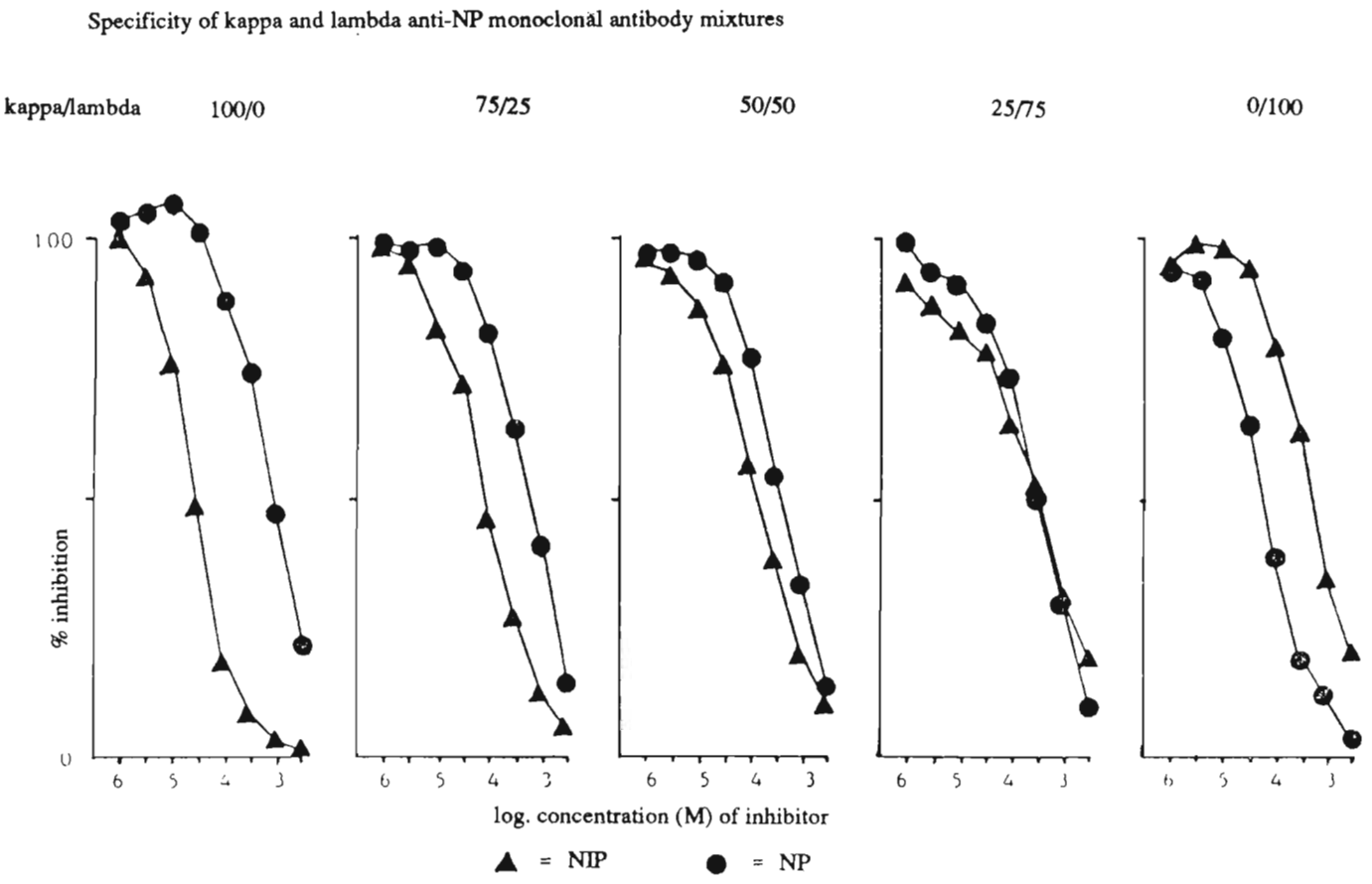

Figure 3. **Inhibition assay of various kappa and lambda bearing anti- NP mAbs mixtures. 4E9 and A6 mAbs mixtures with different ratios were subjected to ELISA/RIA inhibition assay as described in Fig.1. It is clearly observed that the mixture becomes homoclitic with the increase of kappa/lambda ratio.**

Relation between mRNA structure and specificity and affinity of anti-NP mAbs

mAbs*	Isotype	Gene arrangements¶ H chain V	D	J	L chain V	J	Number of somatic mutations¶ V_H	V_L	Specificity*	Affinity ($K_a M^{-1}$)# NP-Gly	NP Cap	NIP Cap
Early :												
B4	μ ,λ_1								ϕ^- > ØH NXP>NP	$2,3x10^5$	$1.8x10^6$	$5,1X10^7$
A1	γ_1 ,λ_1	186	DFL16.1	J_2	V_{λ_1}	J_{λ_1}	0	0	ϕ^- > ØH NXP>NP		$2,5x10^4$	
A6	γ_1 ,λ_1	186	DFL16.1	J_2	V_{λ_1}	J_{λ_1}			ϕ^- > ØH NXP>NP	$1.8x10^5$	$3,4x10^6$	$3,0x10^7$
F8	γ_1 ,λ_1	186	SP2-3	J_4	V_{λ_1}	J_{λ_1}	0	0	ϕ^- > ØH NXP>NP		$2,8x10^5$	
2G2	μ ,κ								ϕ^- > ØH NXP<NP	1 $x10^5$	$1,1x10^6$	
4E9	γ_1 ,κ								ϕ^- > ØH NXP<NP		7 $x10^5$	
10F1	γ_{2a},κ								ϕ^- > ØH NXP<NP		$7,0x10^5$	
Late :												
H1	γ_{2a},λ_1	186	DFL16.1	J_2	V_{λ_1}	J_{λ_1}			ϕ^- > ØH NXP>NP		$4,7x10^6$	$3,0x10^7$
G6	γ_1 ,λ_1	186		J_2	V_{λ_1}	J_{λ_1}	2	3	ϕ^- > ØH NXP>NP		$8,7x10^6$	
g2	γ_1 ,λ_1	186	DFL16.2	J_2	V_{λ_1}	J_{λ_1}	3	6	ϕ^- > ØH NXP>NP		$9,7x10^5$	
E3	γ_{2a},λ_1	186	DFL16.1	J_2	V_{λ_1}	J_{λ_1}	7	16	ϕ^- = ØH NXP>NP	5 $x10^8$		
C6	γ_1 ,λ_1	186	DFL16.1	J_2	V_{λ_1}	J_{λ_1}	4	4	ϕ^- = ØH NXP>NP	1 $x10^8$		
A2	γ_1 ,λ_1				V_{λ_1}	J_{λ_1}			ϕ^- = ØH NXP>NP		$2,8x10^7$	
B10	γ_1 ,λ_1				V_{λ_1}	J_{λ_1}			ϕ^- = ØH NXP>NP		$7,6x10^7$	

Table 1. **mAbs obtained at early and late stages were isotyped using standard immunodiffusion techniques. The ability to bind both forms of NP hapten and the homocliticity were examined to determine its specificity. The affinity was examined using flurorescence quenching method. Sequence of its immunoglobulin genes was obtained by either direct method from mRNA or after cloning its cDNA in to lambda-gt11 and M13 (for details see ref. 2). Number of somatic mutation does influence the ability to recognize both forms of NP hapten but is not directly related to the homocliticity, which is more related to the isotype (light chain).**

to clarify how and when the production switch from lambda to kappa bearing anti-NP antibody occurs. This study will be of importance in the study of immune responses to a given antigen, for example in vaccine development, production of high quality antibody, development and treatment of diseases, etc.

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QUESTIONS AND ANSWERS :

1. Question: Do you study about a monoclonal expansion system to look separately (only Kappa and only lamda) ?

   Answer : Yes we do. A hybridoma can get different specificity after being cloned many times. Also it can change the class (e.g. IgM to IgD).